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(54) REMEDIES FOR DISEASES CAUSED BY INSULIN RESISTANCE

(57) The present invention relates to a remedy for diseases caused by insulin resistance, which comprises, as an active ingredient, a substance exhibiting activity for inhibiting the binding of the full-length insulin receptor substrate-1 (IRS-1) or insulin receptor substrate-2 (IRS-2) or a portion of the same to the full-length 14-3-3 protein or a portion of the same; and to a screening method for a remedy for diseases caused by insulin resistance, which comprises assaying the activity for inhibiting the binding.

By means of the screening method, there can be obtained a remedy for diseases caused by insulin resistance, such as diabetes, diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart diseases, ischemic brain disorders, and peripheral arterial embolism.

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Description

Technical Field

[0001] The present invention relates to a drug, particularly a remedy for diseases caused by insulin resistance, such as diabetes, as well as to a screening method for the remedy.

Background Art

[0002] Insulin is a hormone which regulates the concentration of blood sugar and blood lipid through the promotion of glucose and lipid intake into cells and utilization and storage of them. Insulin resistance indicates the condition in which insulin does not act normally on cells, and this condition causes elevation of the concentration of blood sugar or blood lipid. Examples of diseases caused by insulin resistance include diabetes, diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart diseases, ischemic brain disorders, and peripheral arterial embolism (Tamio Teramoto, et al., (1995) Biomedicine & Therapeutics 29, 8-96). The cause of insulin resistance has not yet been fully elucidated, and causal therapy thereof has not been developed.

[0003] Recently, abnormality of intracellular signal transduction induced by insulin has become of interest as a cause of insulin resistance. In signal transduction of insulin, the first response induced by insulin is activation of insulin receptor tyrosine kinase. Subsequently, several intracellular substrates including insulin receptor substrate-1 (IRS-1) (Sun, X. et al., (1991) Nature 352, 73-77) and insulin receptor substrate-2 (IRS-2) (Sun, X. et al., (1995) Nature 377, 173-177) are phosphorylated. IRS-1 and IRS-2 have potential tyrosine-phosphorylated sites in amounts of 21 and 23, respectively, and they function as "docking protein" which transmits insulin signals to several proteins having Src-homology 2 domains (SH2-protein) (Sun, X. et al., (1993) Mol. Cell. Biol. 13, 7418-7428).

[0004] However, the function of IRS-1 and IRS-2 relating to insulin signal transduction in the aforementioned action is not necessarily fully elucidated, and elucidation of novel function thereof and development of drugs on the basis of the function are demanded.

[0005] An object of the present invention is to elucidate novel function of IRS-1 and IRS-2, and to provide a drug based on the function.

Disclosure of the Invention

[0006] In view of the foregoing, the present inventors have focused on the relation between IRS-1 or IRS-2 and 14-3-3 protein.

[0007] 14-3-3 Protein is widely distributed in eucaryotes such as animals, plants, and yeast, and is a protein family which is supposed to act as a regulatory factor by binding to a particular target protein in a variety of signal transductions depending on phosphorylation and dephosphorylation of proteins (Fumiko Shinkai, et al., (1996) Protein Nucleic Acid Enzyme 41, 313-326). Recently, it has been reported that 14-3-3 protein binds to phosphatidylinositol 3-kinase (PI3K) and inhibits its activity in T lymphocytes (Bonnefoy-Berard, N. et al., (1995) Proc. Natl. Acad. Sci. 92, 10142-10146). PI3K plays an important role in signal transduction of insulin (Masato Kasuga, (1996) Saishin-Igaku 51, 1564-1572), and thus 14-3-3 protein has been supposed to effect some type of regulation against signal transduction of insulin (Humiko Shinkai, et al., (1996) Protein Nucleic Acid Enzyme 41, 313-326). In addition, very recently, it has been reported that the ε isoform of 14-3-3 protein binds to IRS-1, but the physiological significance has not been elucidated (Craparo. A. (1997) J. Biol. Chem. 272. 11663-11669).

[0008] The present inventors have performed extensive studies on the relation between 14-3-3 protein and IRS-1 or IRS-2; have elucidated that IRS-1 or IRS-2 binds to 14-3-3 protein at a particular site and that the binding effects negative regulation against insulin signal transduction; and have found that a substance inhibiting the binding is useful for a remedy for diseases caused by insulin resistance. The present invention has been accomplished on the basis of these findings.

[0009] Accordingly, the present invention provides a remedy for diseases caused by insulin resistance, which comprises, as an active ingredient, a substance exhibiting activity for inhibiting the binding of the full-length IRS-1 or IRS-2 or a portion of the same to the full-length 14-3-3 protein or a portion of the same.

[0010] The present invention also provides a screening method for a remedy for diseases caused by insulin resistance, which comprises assaying activity for inhibiting the binding of the full-length IRS-1 or IRS-2 or a portion of the same to the full-length 14-3-3 protein or a portion of the same.

[0011] The present invention also provides a pharmaceutical composition for diseases caused by insulin resistance, which comprises a substance exhibiting activity for inhibiting the binding of the full-length IRS-1 or IRS-2 or a portion of the same to the full-length 14-3-3 protein or a portion of the same, and a pharmaceutically acceptable carrier.

[0012] The present invention also provides use of a substance exhibiting activity for inhibiting the binding of th full-length IRS-1 or IRS-2 or a portion of the same to the full-length 14-3-3 protein or a portion of the same for producing a remedy for diseases caused by insulin resistanc .

[0013] The present invention also provides a method for treating diseases caused by insulin resistance, which comprises administering to a patient in need thereof an effective dos of a substance exhibiting activity for inhibiting the binding of the full-length IRS-1 or IRS-2 or a portion of the same to the full-length 14-3-3 protein or a portion of the same.

Best Mode for Carrying Out the Invention

[0014] Active ingredients of the remedy of the present invention include a substance exhibiting activity for inhibiting the binding of the full-length IRS-1 or IRS-2 or a portion of the same to the full-length 14-3-3 protein or a portion of the same in screening for assaying the inhibiting activity.

[0015] As described below, the present inventors were the first to elucidate that the binding of 14-3-3 protein to IRS-1 or IRS-2 effects negative regulation against insulin signal transduction.

[0016] Firstly, in order to identify a unique protein that binds to IRS-1, the present inventors used ^{32}P-labeled recombinant IRS-1 as a probe in order to screen a cDNA library derived from human heart, to thereby obtain two isoforms (ε and ζ) which belong to a 14-3-3 protein family. In addition, they found that 14-3-3 protein associates with IRS-1 in L6 muscular cells. HepG2 hepatoma cells, and Chinese hamster ovary cells, in which IRS-1 is overexpressed by means of an adenovirus expression system, as well as in the brain tissue of cow in a natural state.

[0017] The present inventors also elucidated that 14-3-3 protein associates with IRS-1 or IRS-2 in SF9 cells in which 14-3-3 protein and IRS-1 or IRS-2 are overexpressed by means of a baculovirus expression system.

[0018] The present inventors also elucidated, by use of HepG2 hepatoma cells in which IRS-1 is overexpressed in the same manner as described above, that the amount of 14-3-3 protein binding to IRS-1 is not changed by insulin stimulation, and that the amount is significantly increased by okadaic acid, which is an inhibitor of serine/threonine phosphatase.

[0019] The present inventors also elucidated that IRS-1 has three putative binding sites (Ser-270, Ser-374, and Ser-641) for 14-3-3 protein, on the basis of the finding that, in a cell lysate of L6 muscular cells, the binding of IRS-1 to 14-3-3 protein fused with glutathione S-transferase (GST) is inhibited by three types of 15-residue oligopeptide shown in sequence Nos. 2-4 which contains a serine residue and several amino acid residues in the vicinity of it corresponding to the amino acid sequence of IRS-1, and the serine residue is phosphorylated. Of the above three binding sites, the motif around of Ser-270 are located in the phosphotyrosine binding domain (PTB domain) of IRS-1, and the domain is known to play an important role in interaction with insulin receptors (Wolf, G. (1995) J. Biol. Chem. 270, 27407-27410). The present inventors elucidated that, in practice, truncated IRS-1 containing the PTB domain and 205 amino acids adjacent to its C-terminal side associates with GST-fused 14-3-3 protein, by overexpressing the IRS-1 in HepG2 hepatoma cells by means of an adenovirus expression system.

[0020] In addition, the present inventors found that IRS-1 that has been coprecipitated with an antibody against 14-3-3 protein is insusceptible to phosphorylation of serine and tyrosine residues by insulin stimulation as compared with IRS-1 that has been coprecipitated with an antibody against IRS-1, by analyzing the effect of the binding of 14-3-3 protein to IRS-1 on phosphorylation of IRS-1 induced by insulin, and analyzing phosphorylated amino acids in a HepG2 hepatoma cell in which IRS-1 is overexpressed by means of an adenovirus expression system. As described above, it was elucidated that 14-3-3 protein effects negative regulation against insulin signal transduction by inhibiting the association of insulin receptors with IRS-1.

[0021] Therefore, abnormal promotion of the binding of 14-3-3 protein to IRS-1 or IRS-2 is a primary cause for insulin resistance, and thus insulin resistance may be suppressed and diseases caused by insulin resistance may be treated by inhibiting, suppressing, and dissociating the binding. In order to inhibit the binding, direct inhibition may be effected against the binding of 14-3-3 protein to IRS-1 or IRS-2. Alternatively, indirect inhibition may be effected; for example, phosphorylation of a particular serine residue in the amino acid sequence of IRS-1 or IRS-2, which phosphorylation is considered to play an important role in the binding of 14-3-3 protein to IRS-1 or IRS-2, may be inhibited, or dephosphorylation may be promoted.

[0022] The full-length IRS-1 or IRS-2 or a portion of the same used in the present invention may be obtained, for example, by means of the following procedure: cDNA coding for the full-length IRS-1 or IRS-2 or a portion of the same is introduced into baculovirus by means of known methods, and the full-length IRS-1 or IRS-2 or a portion of the same is isolated from the insect cells infected with the virus and purified by means of known methods. The amino acid sequence of IRS-1 is shown in sequence No. 1. When a portion of IRS-1 or IRS-2 is oligopeptide, the portion may be synthesized by means of known peptide synthesis methods. A portion of IRS-1 or IRS-2 may be peptides containing a serine residue in the amino acid sequence of IRS-1 or IRS-2, or phosphorylated products of the peptides. Preferably, a portion of IRS-1 or IRS-2 may be peptid containing the PTB domain (amino acid 161-517 in sequence No. 1 in the

case of IRS-1, the amino acid sequence in sequence No. 5 in the case of IRS-2 (corresponding to amino acid 196-354 of IRS-2)), more preferably oligopeptides containing Ser-270, Ser-374, or Ser-641 of IRS-1, or phosphorylated products of the peptides. The length of the portion is not limited so long as activity for inhibiting the binding can be assayed with high sensitivity, and the portion may be 5-50 amino acids, preferably 10-30 amino acids, more preferably 15 amino acids containing serine which is phosphorylated.

[0023] The full-length 14-3-3 protein or a portion of the same used in the present invention may be obtained, for example, by means of the following procedure: cDNA coding for the full-length 14-3-3 protein or a portion of the same is introduced into baculovirus by means of known methods, and the full-length 14-3-3 protein or a portion of the same is isolated from the insect cell infected with the virus and purified by means of known methods. A portion of 14-3-3 protein may be the box-1 region which is the binding site to tryptophan hydroxylase (Ichimura, T. et al., (1997) FEBS Lett. 413, 273-276), or a peptide containing the region.

[0024] In order to obtain the full-length IRS-1 or IRS-2 or a portion of the same, and the full-length 14-3-3 protein or a portion of the same, they may be advantageously expressed as a fusion protein in a variety of gene expression systems. Fusion protein expression systems such as those including lactose and glutathione S-transferase may also be used.

[0025] In order to prepare the aforementioned screening system, the full-length labeled IRS-1 or labeled IRS-2 or a portion of the same, or the full-length labeled 14-3-3 protein or a portion of the same is preferably used. ^{125}I or an enzyme which is often used in enzyme immunoassay, such as alkaline phosphatase, is appropriately used for labeling. Such a substance for labeling is bonded to the protein by means of known methods. When the labeled protein is not used, a primary antibody specific to the unlabeled protein and a secondary antibody which is labeled and recognizes the primary antibody are necessary. The primary and secondary antibodies may be commercially available ones.

[0026] Next will be described a preferred embodiment for effecting screening for a substance exhibiting activity for inhibiting the binding of 14-3-3 protein to IRS-1 or IRS-2 by means of the above-described system. Firstly, IRS-1 or IRS-2 (the full length or a portion thereof), or 14-3-3 protein (the full length or a portion thereof) is prepared by immobilization thereof onto a plastic material (a microplate or beads) by means of a known method. Subsequently, the other protein to be bonded which is labeled is dissolved in an appropriate buffer, and the resultant solution is added to each well of a microplate (when a microplate is used) or to test tubes containing the beads (when beads are used). A test compound is also added thereto. Independently, a solution containing a very large amount of unlabeled protein is prepared in order to determine the amount of non-specific binding (NSB). The solution containing the labeled protein is incubated under appropriate conditions, and the material (each well of the microplate, or the beads) is washed with the buffer. The amount of the labels attached to the protein binding to the well or beads is measured by means of known methods. When the labeled protein is not used, the solution containing the unlabeled protein is incubated in the same manner, an antibody specific to the unlabeled protein (the primary antibody) is added, and the solution is incubated under appropriate conditions. Furthermore, the secondary antibody which is labeled and recognizes the primary antibody is added, and the solution is incubated under appropriate conditions. Thereafter, the substrate is washed with the buffer, and the amount of labels attached to the protein binding to the substrate is measured in the same manner. When the value of "Bo - NSB"—which is obtained by subtracting NSB from the amount of labels attached to the protein to be bonded in the absence of a binding-inhibitory substance (Bo)—is regarded as 100%, a test compound providing the value (amount of specific binding) of 10% or less may be chosen as a substance exhibiting activity for inhibiting the binding.

[0027] A substance source which is considered to exhibit the binding inhibitory activity may be tested by means of the above-described screening system. Examples of such a substance source include synthetic peptides, low-molecular organic compounds, and natural products, preferably substances having applicability as drugs. Specific examples include combinatorial libraries of different chemical substances and synthetic peptide libraries.

[0028] A substance exhibiting the binding inhibitory activity which is obtained by means of the above-described screening suppresses negative regulation against insulin signal transduction in cells. Therefore, the substance is useful for producing a remedy for diseases caused by insulin resistance. Examples of such diseases include diabetes, diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart diseases, ischemic brain disorders, and peripheral arterial embolism.

[0029] A dosage of the remedy of the present invention depends on the age, sex, and pathological condition of a patient, and is 5 mg-2 g per adult per day, preferably 50-100 mg as reduced to an active ingredient. The aforementioned dosage per day may be administered in a single portion once a day, or in divided portions 2-3 times a day. If necessary, a dosage per day may exceed the aforementioned dosage.

[0030] No particular limitation is imposed on the administration method and the dosage form of the remedy of the present invention, and any dosage form suitable for an administration method may be obtained by means of a conventionally used technique for preparing products.

[0031] Examples of products for oral administration includ tablets, powders, granules, capsules, solutions, syrups, elixirs, and oily or aqueous suspensions.

[0032] For preparation of injections, a solution may be stored in a container and freez -dried, to thereby provide a solid product, and the solid product may be prepared into an injection just before use. If necessary, th product may contain a stabilizer, a pres rvative, and a solubiliz r. A single dosage of the injection product may be stored in a container, or a plurality of dosages may be stored in the same container.

[0033] Examples of external-use products include soluti ns, suspensions, emulsions, ointments, gels, creams, lotions, and sprays.

[0034] Solid products may contain pharmaceutically acceptable additives together with an active ingredient. If necessary, the remedy may optionally contain fillers, expanders, binders, disintegrants, dissolution-promoting agents, humectants, and lubricants, to thereby prepare products.

[0035] Examples of liquid products include solutions, suspensions, and emulsions, and the products may contain additives such as suspending agents and emulsifying agents.

Examples

[0036] The present invention will next be described in more detail by way of examples, which should not be construed as limiting the invention thereto. Example 1 (Method for screening inhibitors for the binding of 14-3-3 protein to IRS-1 or IRS-2 characterized by employing the full-length IRS-1 or IRS-2 or a portion of the same and the full-length 14-3-3 protein or a portion of the same)

[0037] A solution containing a portion of human IRS-1, i.e., a portion including the PTB domain (amino acids 161-517) (1 μg/ml, pH 8.0, 50 mM K_2PO_4) (100 μl) is added to each well of a 96-well microplate, and the plate is allowed to stand at room temperature for one hour, to thereby cause the human IRS-1 to be fixed onto the walls of the wells. The solution in each well is removed and the well is washed three times with a buffer (50 mM HEPES, 150 mM NaCl, 0.1% Triton X-100) (300 μl). Subsequently, a buffer solution containing 0.5% bovine serum albumin (BSA) (300 μl) is added to each well and the plate is allowed to stand at room temperature for one hour, to thereby effect blocking. The solution is removed, and each well is washed three times with a buffer (300 μl). Next, a buffer solution containing full-length human 14-3-3 protein (1 μg/ml) (50 μl) and a buffer containing a target sample for screening (50 μl) are simultaneously added to the well and allowed to stand at room temperature for two hours. Independently, a buffer (50 μl) not containing the sample is added to another well which has been treated in the same manner as described above, and allowed to stand, in order to measure the amount of maximum binding (Bo). In order to measure the amount of non-specific binding (NSB), a well to which a portion including the PTB region has not been fixed is subjected to the above-described treatment after blocking, and a buffer solution containing human 14-3-3 protein (50 μl) and a buffer (50 μl) are added to the well, and allowed to stand. Solutions in the above wells are removed, and the wells are washed three times with a buffer (300 μl). A buffer solution containing an anti-human 14-3-3 rabbit polyclonal antibody (Santa Cruz Biotechnology) (0.2 μg/ml) (100 μl) is added to each of the above wells, and the plate is allowed to stand at room temperature for one hour. The solution is removed and the well is washed three times with a buffer (300 μl). Subsequently, a buffer solution containing an alkaline-phosphatase-labeled anti-rabbit IgG goat polyclonal antibody (Linco) (1 μg/ml) (100 μl) is added to each of the wells, and allowed to stand at room temperature for one hour. The solution is removed and the well is washed three times with a buffer (300 μl). After a p-nitrophenyl phosphate solution (1 mg/ml, 1 M diethanolamine) (100 μl) is added to each of the wells, the well is allowed to stand at 37°C for 30 minutes, and a 5% EDTA aqueous solution (100 μl) is added, to thereby terminate the reaction. The absorbance of the resultant product in each well is measured at a wavelength of 405 nm, and the absorbance is regarded as the amount of binding. The binding amount when a sample is added is represented by B. A percentage of binding inhibition by the sample is obtained by the following formula. When the percentage is 10% or less, the sample is selected as a candidate for a substance used in the present invention.

$$\text{Binding inhibitory percentage (\%)} = (1-(B-NSB)/(Bo-NSB)) \times 100$$

Example 2

[0038] Three types of synthetic peptide shown in sequence Nos. 2-4 were obtained by use of reagents for peptide synthesis (for example, a peptide block, peptides and amino acids with protective groups, and phosphorylated serine; products of PerkinElmer) by means of a peptide synthesizer (Model: PerkinElmer 433A, product of PerkinElmer). Activity of these peptides ware assayed by means of the binding inhibition screening system described in Example 1. These synthetic peptides exhibit the binding inhibitory activity.

Industrial Applicability

[0039] By means of the screening method of the present invention, there can be obtained a remedy for diseases

caused by insulin resistance, such as diabetes, diabetic microangiopathies (diabetic nephropathy, diabetic neuropathy, and diabetic retinopathy), impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypert nsion, obesity, ischemic heart diseases, ischemic brain disorders, and peripheral arterial embolism.

Claims

1. A remedy for a disease caused by insulin resistance, which comprises, as an active ingredient, a substance exhibiting activity for inhibiting the binding of a full-length or a portion of insulin receptor substrate-1 (IRS-1) or a full-length or a portion of insulin receptor substrate-2 (IRS-2) to a full-length or a portion of 14-3-3 protein.

2. A remedy according to claim 1, wherein the portion of IRS-1 or IRS-2 is a serine-residue-containing oligopeptide in the amino acid sequence of IRS-1 or IRS-2, or a phosphorylated product of the oligopeptide.

3. A remedy according to claim 1 or 2, wherein the disease caused by insulin resistance is diabetes, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart disease, ischemic brain disorder, or peripheral arterial embolism.

4. A screening method for a remedy for a disease caused by insulin resistance, which comprises assaying activity for inhibiting the binding of a full-length IRS-1 or IRS-2 or a portion of the same to a full-length 14-3-3 protein or a portion of the same.

5. A screening method according to claim 4, wherein the portion of IRS-1 or IRS-2 is a serine-residue-containing oligopeptide in the amino acid sequence of IRS-1 or IRS-2, or a phosphorylated product of the oligopeptide.

6. A screening method for a remedy for a disease caused by insulin resistance, which comprises assaying activity for inhibiting the binding of a protein containing phosphotyrosine binding domain (PTB domain) of IRS-1 or IRS-2, or a phosphorylated protein to a protein containing a binding site of 14-3-3 protein to tryptophan hydroxylase (the box-1 region).

7. A screening method according to any one of claims 4 to 6, wherein the disease caused by insulin resistance is diabetes, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart disease, ischemic brain disorder, or peripheral arterial embolism.

8. A remedy for a disease caused by insulin resistance, which comprises, as an active ingredient, a substance selected by use of the screening method as described in any one of claims 4 to 7.

9. A pharmaceutical composition for a disease caused by insulin resistance, which comprises a substance exhibiting activity for inhibiting the binding of a full-length IRS-1 or IRS-2 or a portion of the same to a full-length 14-3-3 protein or a portion of the same, and a pharmaceutically acceptable carrier.

10. A pharmaceutical composition according to claim 9, wherein the portion of IRS-1 or IRS-2 is a serine-residue-containing oligopeptide in the amino acid sequence of IRS-1 or IRS-2, or a phosphorylated product of the oligopeptide.

11. A pharmaceutical composition according to claim 9 or 10, wherein the disease caused by insulin resistance is diabetes, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart disease, ischemic brain disorder, or peripheral arterial embolism.

12. Use of a substance exhibiting activity for inhibiting the binding of a full-length IRS-1 or IRS-2 or a portion of the same to a full-length 14-3-3 protein or a portion of the same for producing a remedy for a disease caused by insulin resistance.

13. Use according to claim 12, wherein the portion of IRS-1 or IRS-2 is a serine-residue-containing oligopeptide in the amino acid sequence of IRS-1 or IRS-2, or a phosphorylated product of the oligopeptide.

14. Use according to claim 12 or 13, wherein the disease caused by insulin resistance is diabetes, diabetic nephropa-

thy, diabetic neuropathy, diabetic r tinopathy, impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity, ischemic heart disease, ischemic brain disorder, or peripheral arterial embolism.

15. A treatment method for a disease caused by insulin resistance, which comprises administering to a patient in need thereof an effective amount of a substance exhibiting activity for inhibiting the binding of a full-length IRS-1 or IRS-2 or a portion of the same to a full-length 14-3-3 protein or a portion of the same.

16. A treatment method according to claim 15, wherein the portion of IRS-1 or IRS-2 is a serine-residue-containing oligopeptide in the amino acid sequence of IRS-1 or IRS-2, or a phosphorylated product of the oligopeptide.

17. A treatment method according to claim 15 or 16, wherein the disease caused by insulin resistance is diabetes, diabetic nephropathy, diabetic neuropathy, diabetic retinopathy, impaired glucose tolerance, hyperinsulinemia, hyperlipemia, arteriosclerosis, hypertension, obesity. ischemic heart disease, ischemic brain disorder, or peripheral arterial embolism.

18. A pharmaceutical composition for a disease caused by insulin resistance, which comprises a substance selected by use of the screening method as described in any one of claims 4 to 7 and a pharmaceutically acceptable carrier.

19. Use of a substance selected by use of the screening method as described in any one of claims 4 to 7 for producing a remedy for a disease caused by insulin resistance.

20. A treatment method for a disease caused by insulin resistance, which comprises administering to a patient in need thereof an effective amount of a substance selected by use of the screening method as described in any one of claims 4 to 7.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP98/04293

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[6] A61K38/17, G01N33/15

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[6] A61K38/17, G01N33/15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS (STN), REGISTRY (STN), WPIDS (STN), MEDLINE (STN), BIOTECHABS (STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CRAPARO, A., et al., "14-3-3(ε) Interacts with the Insulin-like Growth Factor I Receptor and Insulin Receptor Substrate I in a Phosphoserine-dependent Manner", J. Biol. Chem., April. 1997, 272(17), pp.11663-11669	1-14, 18, 19
PA	KOSAKI, A., et al., "14-3-3β Protein Associates with Insulin Receptor Substrate 1 and Decreases Insulin-stimulated Phosphatidylinositol 3'-Kinase Activity in 3T3L1 Adipocytes", J. Biol. Chem., Jan. 1998, 273(2), pp.940-944	1-14, 18, 19
PA	OGIHARA, T., et al., "14-3-3 Protein Binds to Insulin Receptor Substrate-1, One of the Binding Sites of Which Is in the Phosphotyrosine Binding Domain", J. Biol. Chem., Oct. 1997, 272(40), pp.25267-25274	1-14, 18, 19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 December, 1998 (07. 12. 98)	15 December, 1998 (15. 12. 98)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP98/04293

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [x] Claims Nos.: 15-17, 20

because they relate to subject matter not required to be searched by this Authority, namely:
The inventions of claims 15 to 17 and 20 pertain to methods for treatment of the human body by therapy.

2. [ ] Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

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